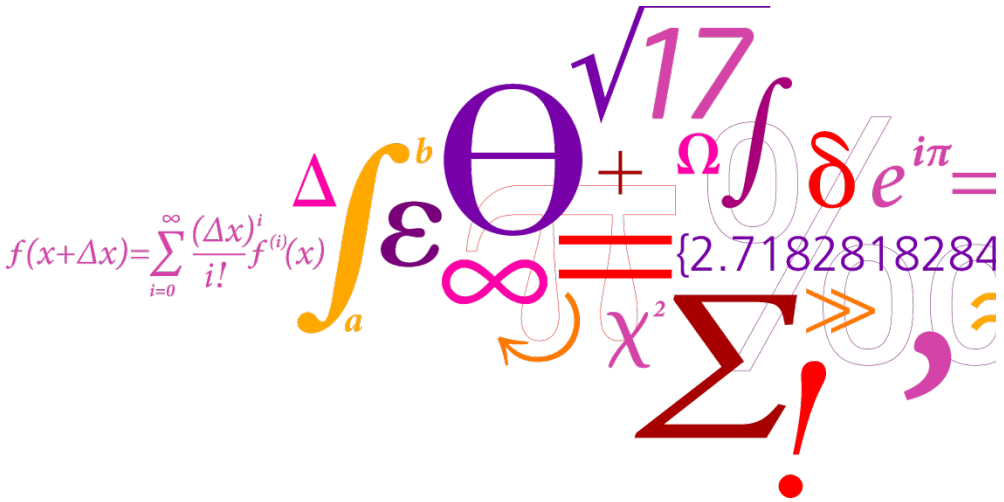
Confocal Microscopy and Atomic Force Microscopy (AFM) of biofilms

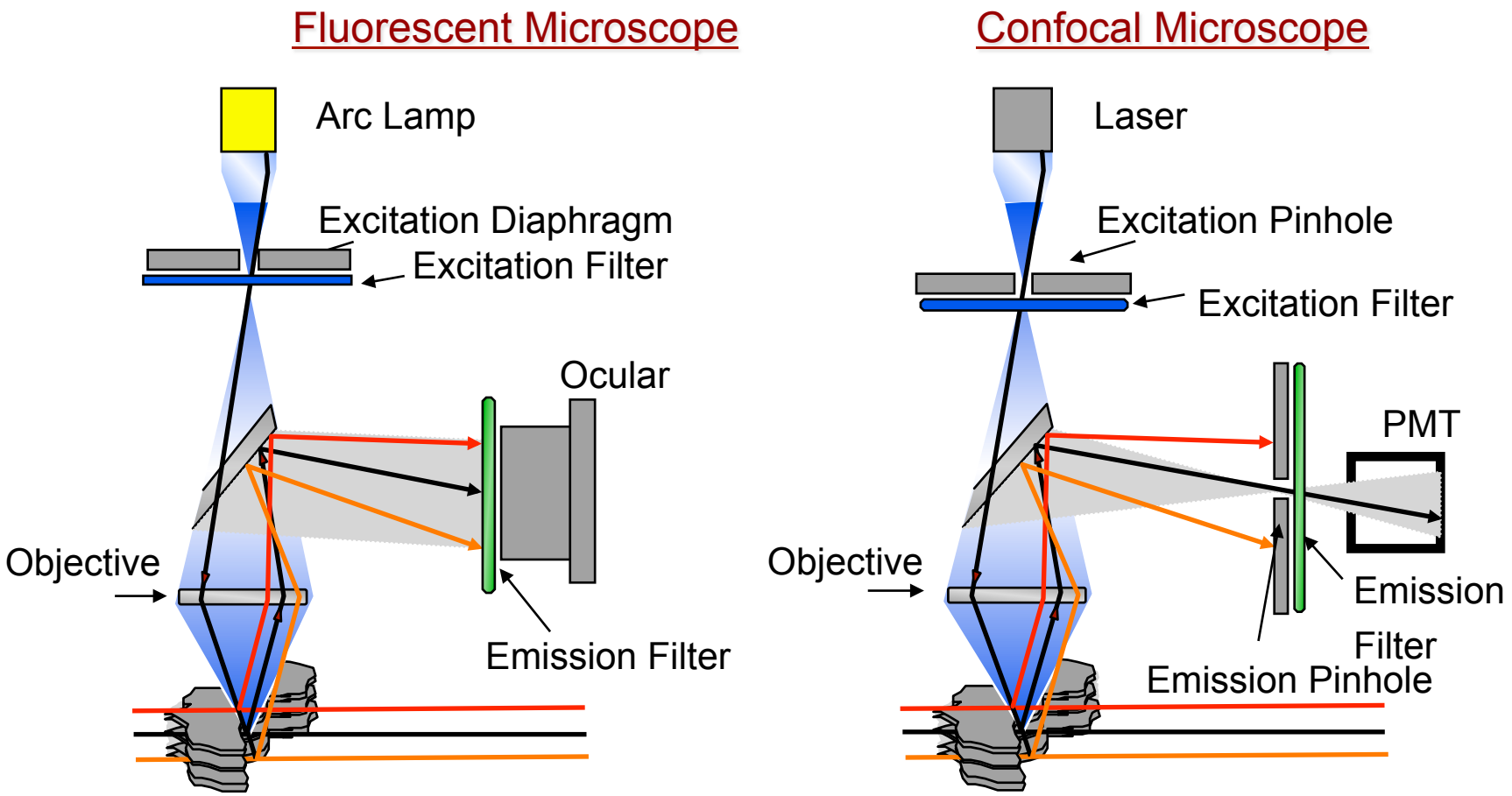
A very brief primer...



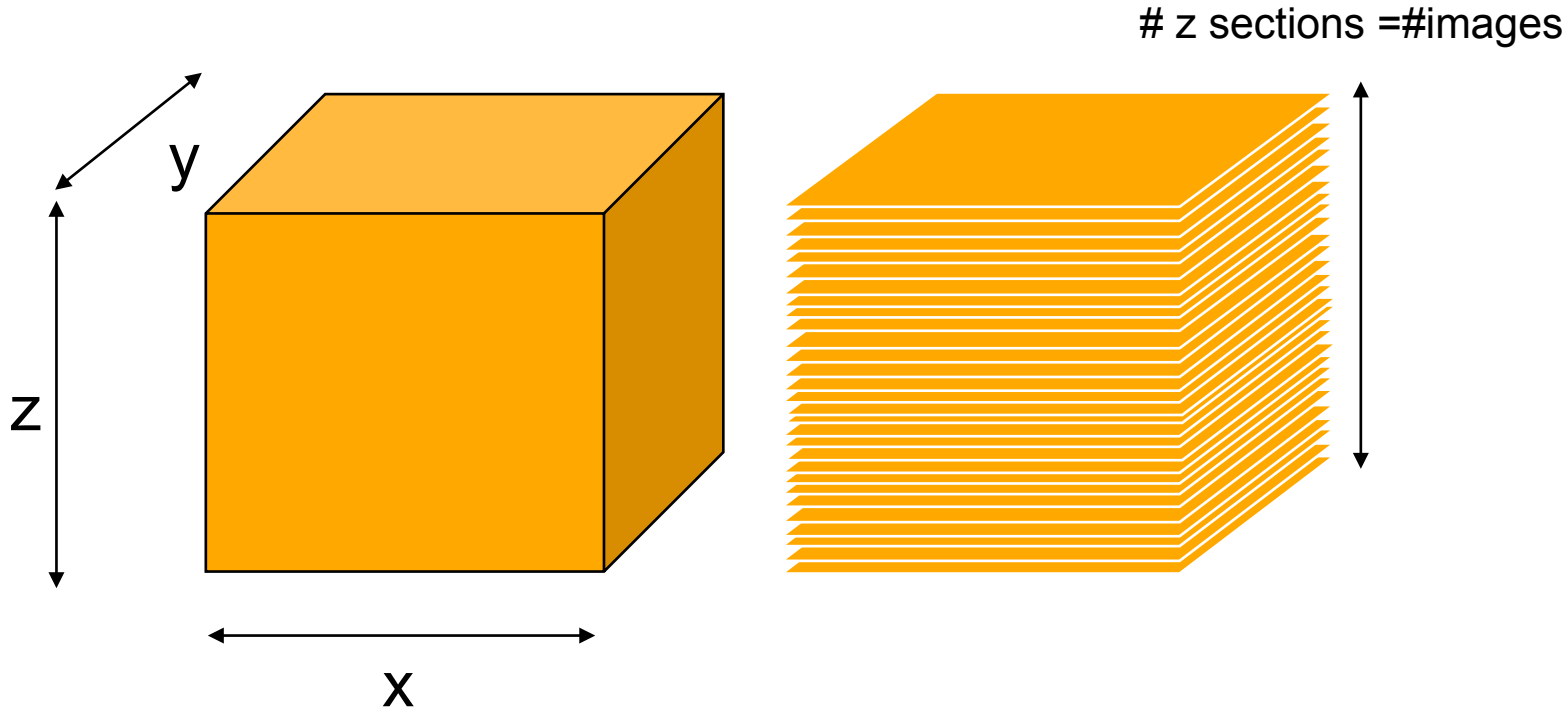
DTU Systems Biology
Department of Systems Biology

Fundamentals of Confocal Microscopy

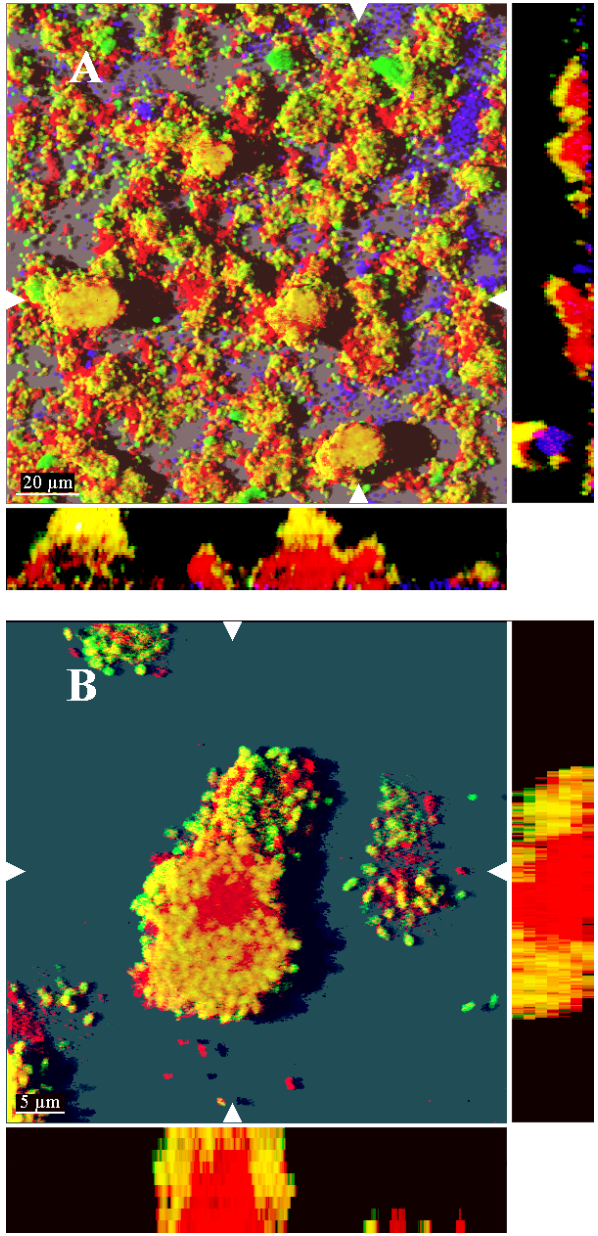
Based on a conventional fluorescence microscope



3D reconstruction



Pseudomonas putida cells mixed with *Acinetobacter* cells in a microbial biofilm

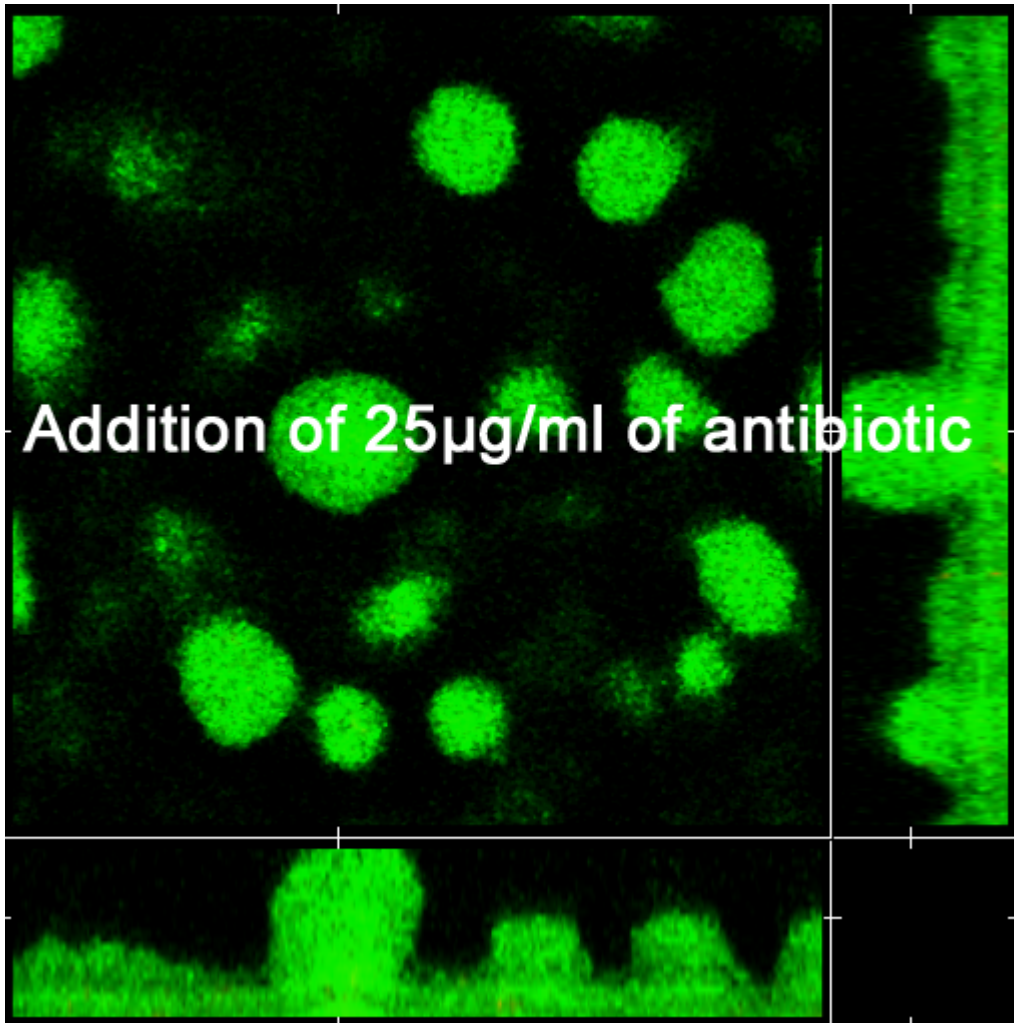


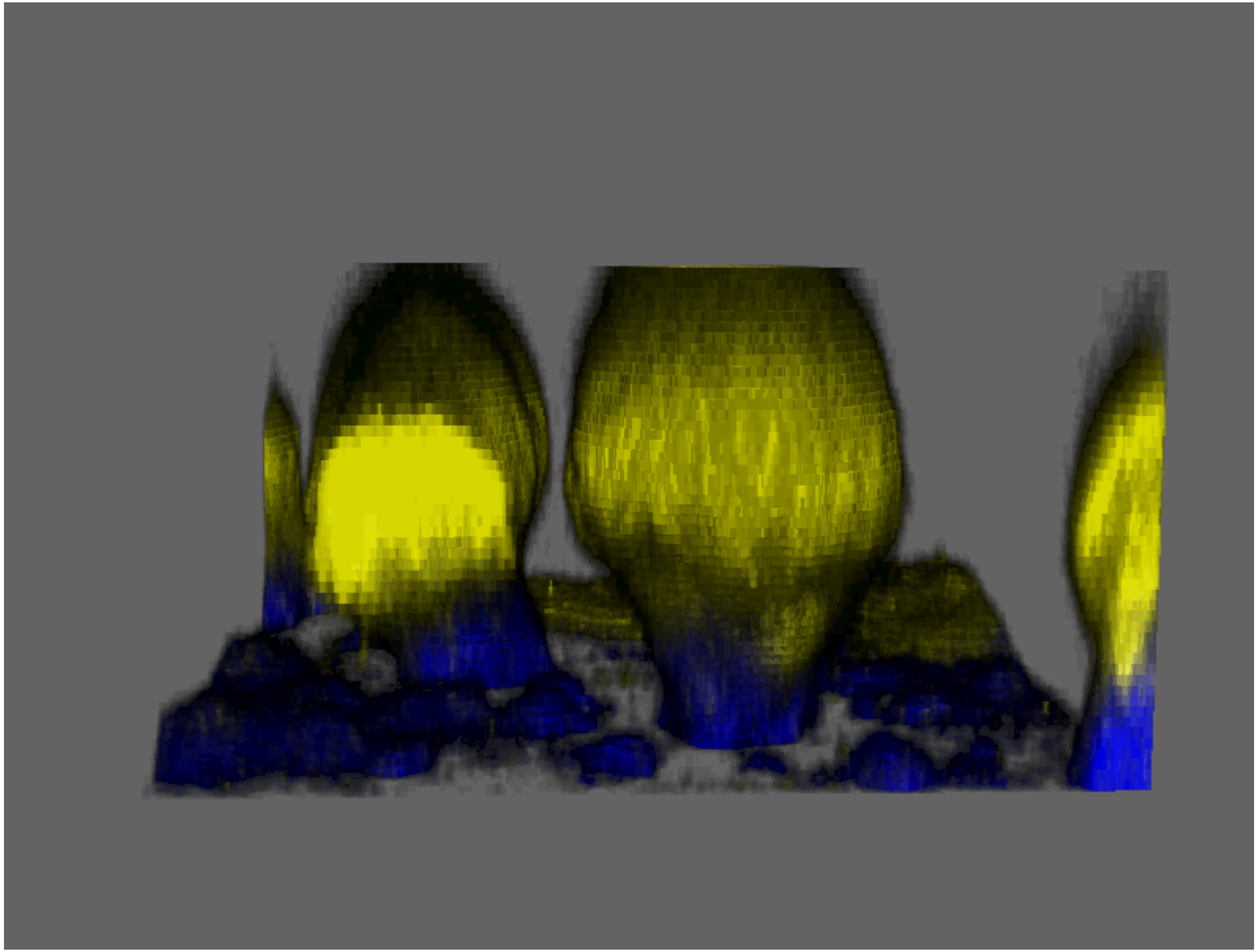
Benefits of Confocal Microscopy

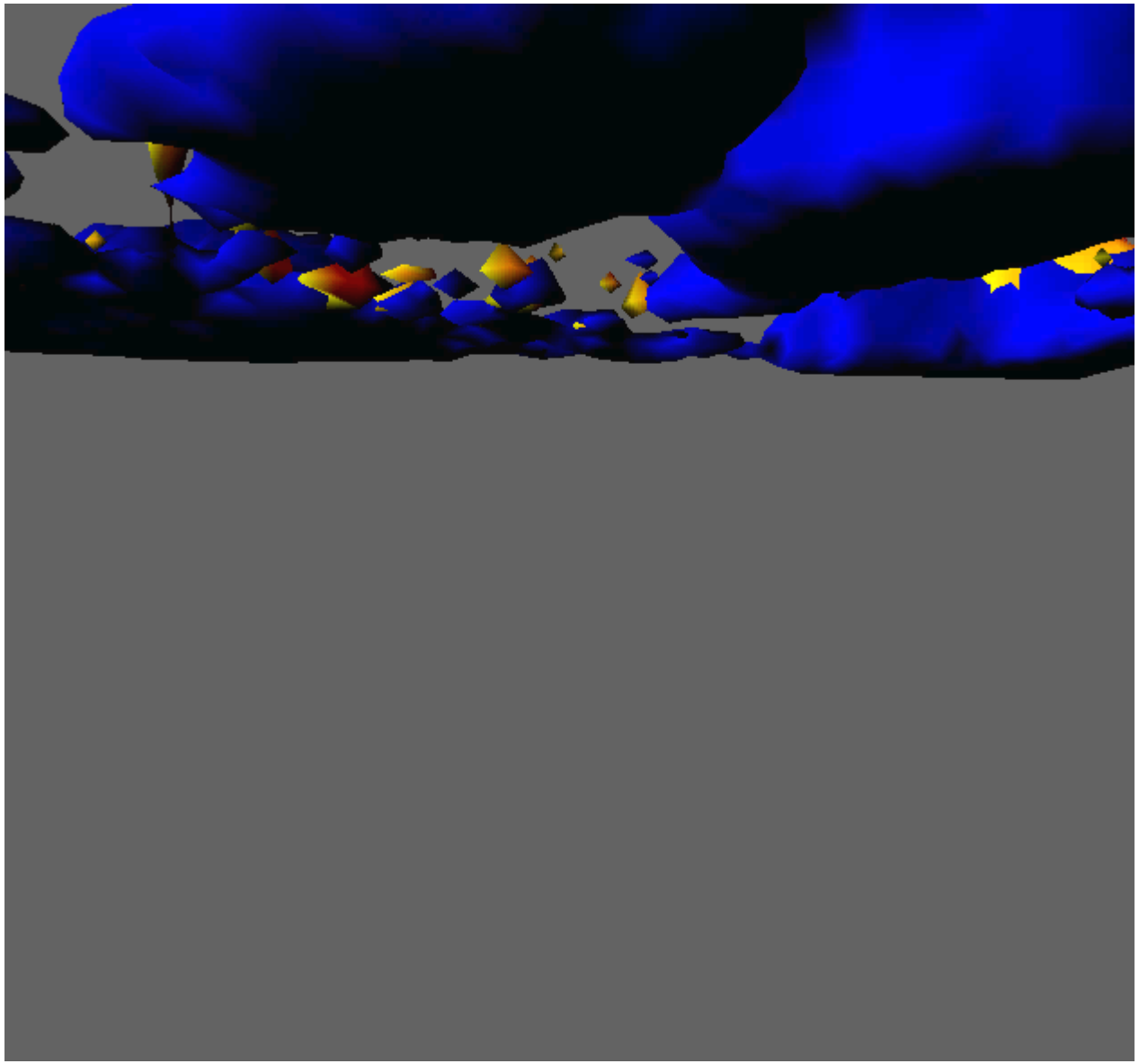
- Reduced blurring of the image from light scattering
- Increased effective resolution
- Improved signal to noise ratio
- Clear examination of thick specimens
- Z-axis scanning (3D-reconstruction possible)
- Magnification can be adjusted electronically
- X-Y resolution: ~200-250 nm (Ernst Abbe)

Disadvantages of Confocal Microscopy

- Requires fluorescent samples
- Uses laser illumination (expensive, few wavelengths)
- Instrument expensive to acquire and run
- Z-resolution typically >500 nm


Addition of 25µg/ml of antibiotic





New developments in confocal microscopy

- **MP (multi-photon) or two-photon confocal microscopy**: Two or more photons from a long wavelength illumination at a time excites a fluorophore. High resolution in Z-axis (practally down to ~200 nm)

- **White Laser (Leica):** A continous wave ”white” laser (tunable from 470-670 nm, with up to 8 lines simultaneously)

- **STED (Stimulated Emission Depletion)(Leica):** A new method where fluourescence is depleted around the area of interest (see next slide)

- **Superresolution structured microscopy (Zeiss):** A methods where images are rotated and combined to create a moire pattern which is deconvolved to create a high res image.

- **Photo-Activated localization microscopy (Zeiss):** Sequential illumination and localization of fluorophores combined with computational reconstruction of high res images.

- **Raman-confocal microscopy (Leica, under development):** Confocal microscope combined with raman spectroscope. Enables localized determination of [changes in] concentrations of metabolites etc.

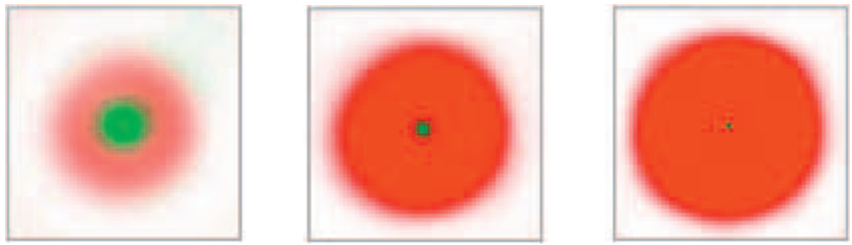
STED (Stimulated Emission Depletion)

- In a Leica TCS STED microscope the sample is illuminated by two pulsed laser beams, tightly synchronized.

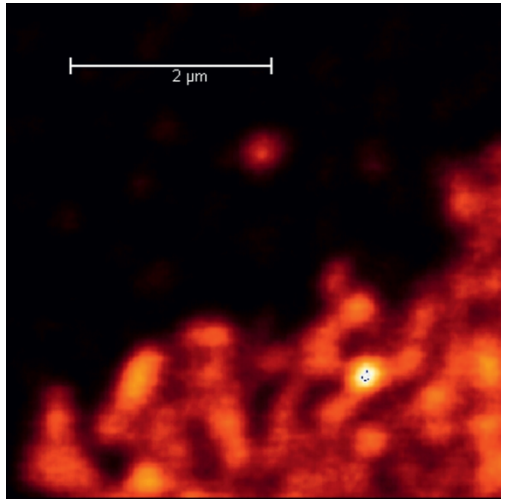
- The 635 nm wavelength excites the fluorophores of the sample the same way a conventional confocal system does. The excitation laser pulses are directly followed by a ring shaped illumination of a Ti:Sapphire Infrared laser 730-780 nm).

- This pulse inhibits/depletes the fluorescence in the outer regions of the illuminated spot.

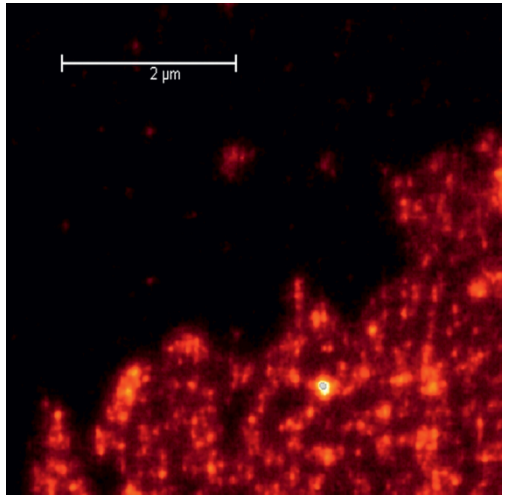
- The result: A smaller fluorescence spot that allows much more accurate scanning than with other methods using focused light. X-Y res: <90 nm



Sample STED image



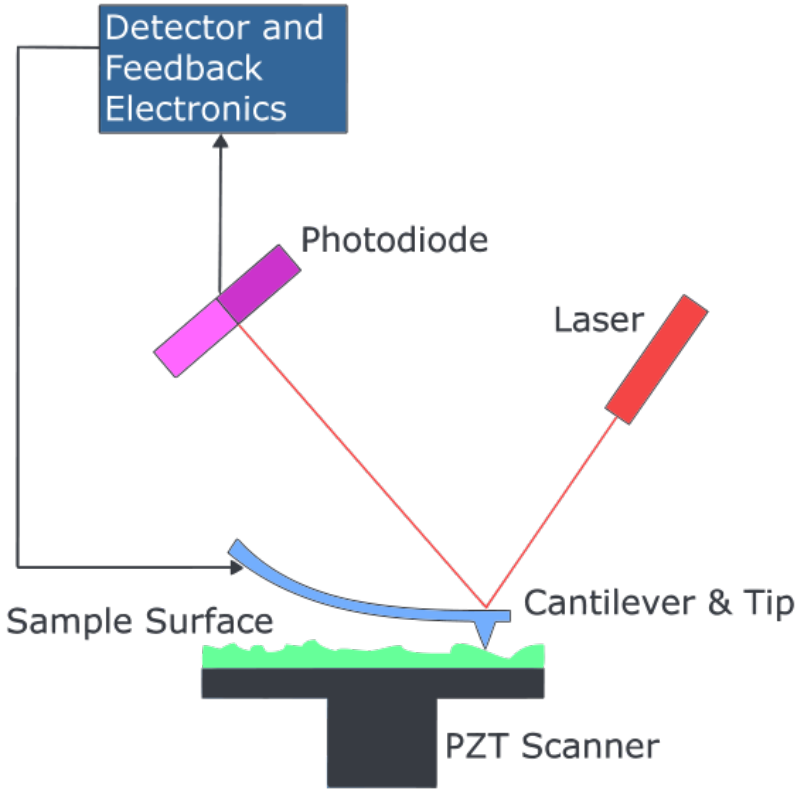
Confocal image



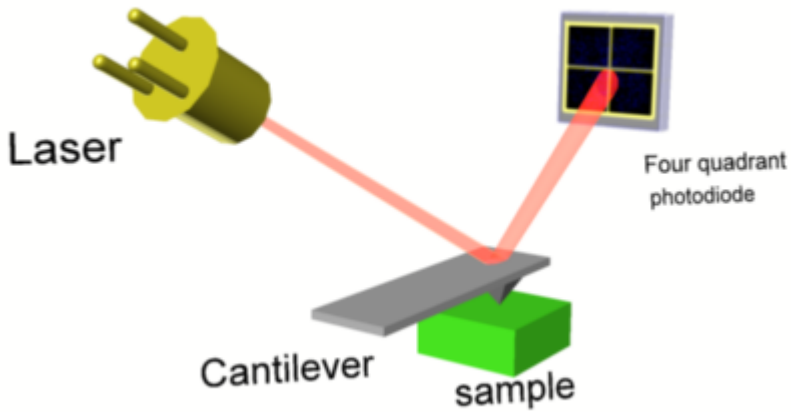
STED image

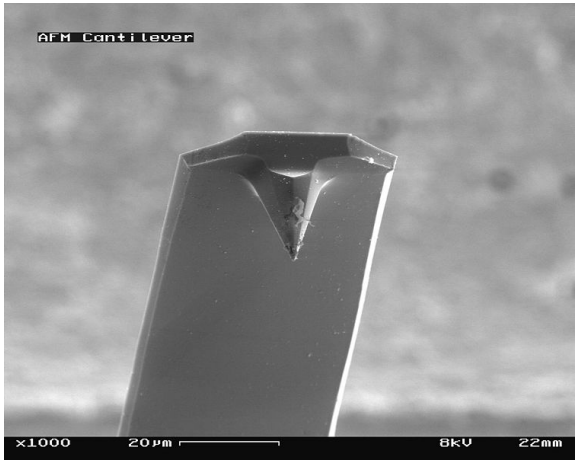
Atomic Force Microscopy (AFM)

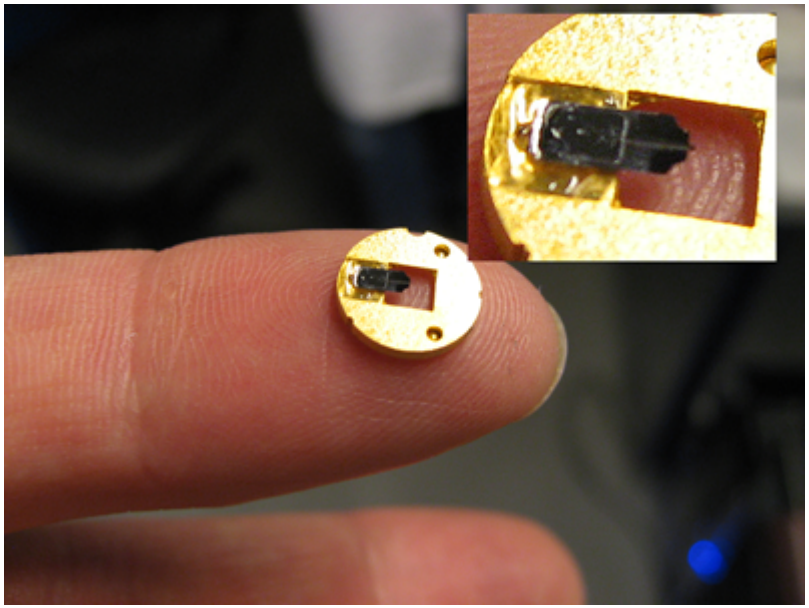
- A method to record the topographic property of a surface
- Physical interaction with the surface is necessary

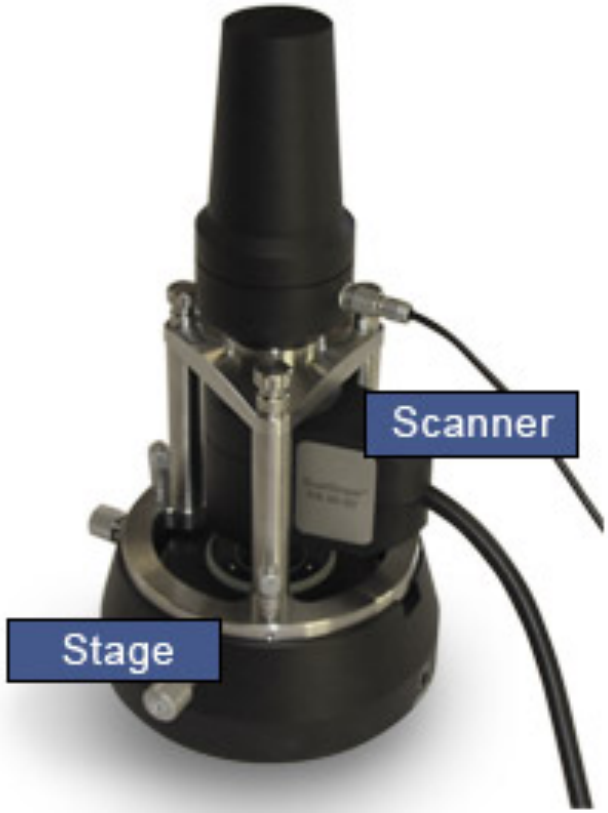


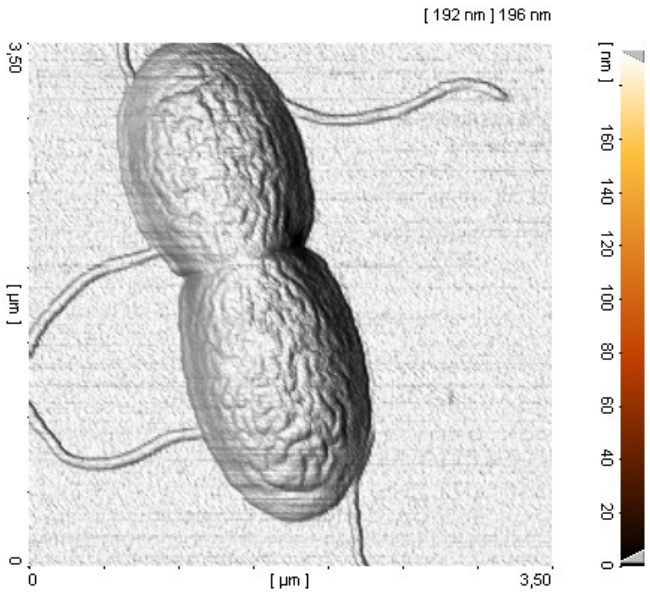
The principle

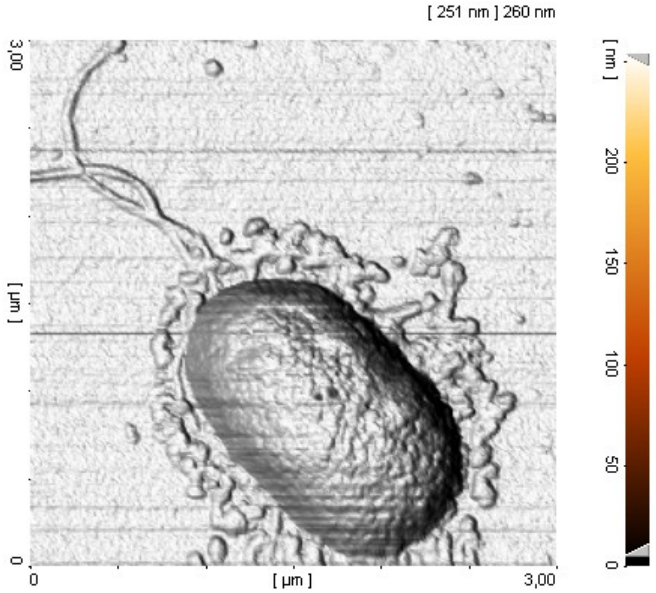



AFM Cantilever
x1000
20µm
8kV
22mm




Scanner
Stage


[192 nm] 196 nm
3,50
[µm]
0
0
[µm]
3,50
[nm]
160
140
120
100
80
60
40
20
0


[251 nm] 260 nm
3,00
[µm]
0
0
[µm]
3,00
[nm]
200
150
100
50
0

Benefits of AFM

- Extemely high resolution (down to atomic level few Å), but typically 10-20 nm)
- No staining required
- Possible to measure attractive/replusive forces

Disadvantages of AFM

- Extremely high resolution (very low ”focal depth”)
- Small image area
- Sample must be ”flat” and tightly fixed
- Very difficult to work in humid or wet environments
- Need direct contact to sample
- Imaging depends on tip shape
- Slow